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# Role of HIV-1 Phenotype in Viral Pathogenesis and Its Relation to Viral Load and CD4+ T-Cell Count

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The predictive value of HIV-1 phenotype in peripheral blood mononuclear cell (PBMC) coculture and the relation among viral phenotype, viral load, and CD4+ T-cell count were examined in two studies. In study A, 132 HIV-1–infected individuals were examined retrospectively for the relation between the result of their initial HIV cultivation in PBMC coculture and survival rate 6 years later. In study B, 176 patients were examined since 1994 for markers of HIV disease progression. HIV-1 phenotype was determined by PBMC cocultivation, viral load by NASBA HIV RNA QT System, and CD4+ T-cell count by flow cytometry. In study A, the percentage of survival for patients with initial negative virus culture was significantly higher (95%) than in patients with nonsyncytia-inducing (NSI) isolates (78%) and syncytia-inducing (SI) isolates (21%) ($P < 0.05$ and $P < 0.0001$, respectively). When SI phenotype was subdivided into moderately cytopathogenic and highly cytopathogenic, significant differences in the rate of survival between these subgroups could be observed (45% vs. 14%; $P < 0.05$). In study B, progression from negative virus culture to the isolation of NSI variants was associated with increasing viral load ($P < 0.0001$) but did not affect CD4+ T-cell count significantly ($P > 0.07$), whereas the switch from NSI to SI virus was accompanied by significant decline of CD4+ T-cells ($P < 0.0001$) but no change in viral load ($P > 0.21$). Thus, isolation and phenotyping of HIV represents an additional striking predictive marker for progression of HIV infection. ***J. Med. Virol. 56:259–263, 1998.***



**KEY WORDS:** viral load; CD4+ T-cell count; PBMC coculture; disease progression; survival rate

## INTRODUCTION

Since the human immunodeficiency virus (HIV) was identified as the causative agent of the acquired immunodeficiency syndrome (AIDS) [Barré-Sinoussi et al., 1983], clinicians and researchers have been looking for predictive markers for disease progression, including clinical, immunological, and virological data. The determination of the number of CD4+ T-cells was found to be a suitable tool for the characterization of the stage of immunodeficiency. However, this surrogate marker failed to provide any information about the viral activity in the organism.

In 1993/1994, it was demonstrated that HIV-1 RNA is detectable in all HIV-1–infected patients by quantitative competitive RT-PCR [Piatak et al., 1993; Bagnarelli et al., 1994]. The investigators also showed that the amount of HIV-1 RNA in plasma varied over a wide range, with significantly higher HIV RNA levels in primary infection and AIDS and substantially lower levels during the stage of clinical latency. Based on these observations, the determination of plasma viral load has become increasingly important for monitoring and characterization of HIV infection. Recently, Mellors et al. [1996] were able to document a strong correlation between the level of viral load during the early stage of clinical latency and the rate of progression to AIDS and death.

Cocultivaton of peripheral blood mononuclear cells (PBMC) isolated from HIV-1–infected patients with PBMC from noninfected blood donors was established [Barré-Sinoussi et al., 1983; Popovic et al., 1984] in order to identify the causative agent of AIDS. Using this technique, highly cytopathic, syncytia-inducing (SI) HIV strains and virus strains showing very low or no cytopathic effect (CPE), i.e., nonsyncytia-inducing (NSI) strains, were isolated [Asjö et al., 1986; Rübsamen-Waigmann et al., 1986; Tersmette et al., 1988; Schneweis et al., 1989]. In a third group of patients, HIV could not be cultured from the PBMC. For a majority of these patients, these findings were constant over several years, whereas in a minority of patients, a

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development from culture negative to the SI phenotype with an intermediate NSI stage was observed [Witt et al., 1996]. Previous studies have shown a good correlation of the HIV phenotype with the CD4+ T-cell count [Schneweis et al., 1990; Koot et al., 1993a; Fouchier et al., 1995; Koot et al., 1996] and with progression to AIDS [Koot et al., 1993b; Richman et al., 1994].

Although there have been investigations on the correlation of CD4+ T-cell count with the viral phenotype or viral load, the relation between viral load and viral phenotype was not yet established. In this study the relation of all three parameters were examined in 176 HIV-1–infected patients. Moreover, in 132 patients the survival rate was examined in relation to the results of virus isolation in order to estimate the clinical relevance of this parameter.

## MATERIALS AND METHODS

### Patients

In study A, a cohort of 132 HIV-1–infected individuals was examined retrospectively for the relation between the time of survival and the results of virus cultivation in PBMC coculture. In study B, 176 HIV-1–infected individuals, including 127 hemophiliacs, were investigated concerning the parameters listed below. The cohort included nucleoside reverse transcriptase inhibitors (NRTI)-treated as well as treatment-naive patients. None of the patients was treated with protease inhibitors.

### Isolation of HIV and Determination of NSI/SI Phenotype

Isolation of HIV from PBMC separated from 20 ml heparin blood using the principles of the standard procedure [Hollinger et al., 1992] was previously described in detail [Schneweis et al., 1990]. Since there were transitions from the NSI to the SI phenotype, five groups of HIV isolates in cell culture were defined: (1) large syncytia after 4 to 6 days of culture (highly cytopathogenic virus); (2) smaller syncytia after more than 7 days (moderately cytopathogenic virus); (3) few and very small syncytia only in subculture (weakly cytopathogenic virus); (4) no syncytia, but p24 antigen-positive supernatants, cellfree transmissible to subculture (noncytopathogenic virus); and (5) no syncytia, p24 antigen-positive supernatants, but virus not cellfree transmissible to subculture (nontransmissible virus).

For the purpose of this investigation, groups 1 and 2 were classified as SI variants and groups 3, 4, and 5 as NSI variants. This classification was in agreement with the genetic characterization of the V3 region of the *env* gene [Witt et al., 1996]. In cases where HIV could not be cultured, virus was termed (6) "nonculturable."

### Detection of HIV-1 p24 Antigen

Culture supernatants were tested for the presence of HIV-1 p24 antigen using the Coulter HIV-1 p24 Antigen Assay (Coulter Immunotech Diagnostics, Krefeld, Germany).

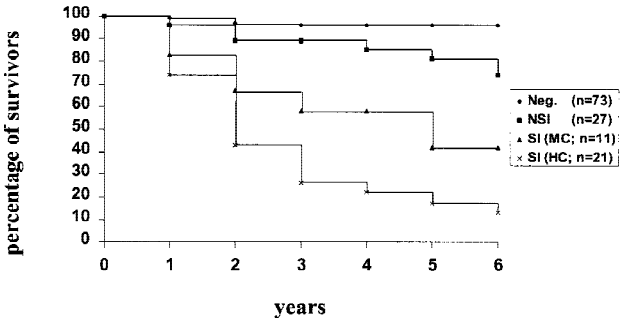


Fig. 1. Correlation of survival time with the results of the initial virus culture. PBMC from 132 HIV-1–infected patients were cocultured with PBMC of healthy blood donors. After 6 years of observation, 96% of the patients with initial negative virus culture still were alive. Only 13% of the patients with a highly cytopathogenic SI virus isolate survived the observation period. Neg. denotes negative virus culture; NSI, nonsyncytia-inducing virus; SI, syncytia-inducing virus; MC, moderately cytopathogenic virus isolate; HC, highly cytopathogenic virus isolate.

### Quantification of Plasma HIV-1 RNA

HIV-1 RNA from serum/plasma samples was quantified using the NASBA HIV-1 RNA QT System (Organon Teknika, Heidelberg, Germany). The detection limit of this assay was 4,000 HIV RNA copies/ml sample since only 100 μl serum/plasma were analyzed. For statistical analysis samples with lower HIV RNA, titers were set as 4,000 copies/ml serum/plasma.

### CD4+ T-Cell Counts

The numbers of CD4+ T-cells were determined by flow cytometry (Coulter, Immunotech Diagnostics, Krefeld, Germany).

### Statistical Analysis

WinStat program version 3.1 was used for the statistical analysis of our data. Spearman ranks correlation was used for the determination of correlations. For the calculation of significances we used the Mann-Whitney U-test.

## RESULTS

In study A, the prognostic value of the HIV-1 phenotype was examined in 132 HIV-1–infected individuals. The results are shown in Figure 1. After 6 years of observation, 95% of all patients with an initially negative virus culture were still alive. The percentage of survival decreased significantly in patients with NSI isolates (78%) and SI isolates (21%) ($P < 0.05$ and $P < 0.0001$, respectively). When SI phenotype was subdivided into highly cytopathogenic and moderately cytopathogenic, significant differences were observed in the rate of survival between these subgroups (14% vs. 45%; $P < 0.05$).

In study B, 344 blood samples from a cohort of 173 HIV-infected patients were examined in relation to the

TABLE I. Parameters of HIV-1 Infection: Relation of HIV Phenotype, Viral Load, and $CD4^+$ T-Cell Count

| HIV phenotype | Mean $CD4^+$ T-cell count | Median $CD4^+$ T-cell count | Mean HIV RNA titer × $10^3$/ml serum | Median HIV RNA titer × $10^3$/ml serum |
|---|---|---|---|---|
| Negative n = 167 | 365 | 336 | 18.8 | <4 |
| NSI n = 84 | 355 | 280 | 96.7 | 35 |
| SI n = 93 | 182 | 120 | 117 | 44 |

HIV phenotype isolated from patients PBMC, viral load in plasma, and CD4+ T-cell count.

Confirming the results of Mellors et al. [1996], a weak negative correlation was found between CD4+ T-cell count and viral load in plasma for these patients ($r = -0.36$).

HIV-1 of the SI phenotype and NSI phenotype was isolated from 93 (27%) and 84 (24%) samples, respectively, whereas in 167 (49%) cases HIV was not culturable using the applied method. In Table I HIV phenotype was compared with CD4+ T-cell count and viral load. Of note is that the CD4+ T-cell counts of samples with negative virus isolation are comparable with the number of CD4+ T-cells found in patients with NSI phenotype (mean: 365 and 355; median: 336 and 280, respectively; $P > 0.07$), whereas the SI phenotype was associated with a significantly lower CD4+ T-cell count (mean: 182; median: 120; $P < 0.0001$). On the other hand, the HIV RNA titers of the SI cohort and the NSI cohort differed not significantly (mean: 117,000 and 96,700; median: 44,000 and 35,000, respectively; $P > 0.21$) and both were significantly higher than the HIV RNA titers in patients with negative virus isolation (mean: 18,800; median: < 4,000; $P < 0.0001$).

Negative results in virus cultivation were preferably associated with a low viral load (Fig. 2A). In patients with less than 4,000 HIV RNA copies/ml serum, only 20% had detectable HIV in cell culture. In contrast, 88% of the patients with HIV RNA titers higher than $10^5$/ml serum harbored culturable virus strains. The detection rate of both NSI and SI virus increased with increasing HIV RNA titers, so that the ratio of NSI to SI virus remained constant (Fig. 2A).

The relation of the results of virus isolation and CD4+ T-cell counts are shown in Fig. 2B. In 79% of the patients with CD4+ T-cell counts below 100/μl blood, HIV could be cultivated from PBMC, whereas the frequencies of detection of HIV in the patient groups with CD4+ T-cell counts 101–200, 201–500 and >500 μl/blood were 45%, 46%, and 35%, respectively. Interestingly, different distribution patterns of the HIV phenotype were observed concerning the number of CD4+ T-cells. The rate of detection of the SI phenotype increased significantly, concomitantly with decreasing CD4+ T-cells (Fig. 2B), whereas NSI virus was isolated with similar frequencies within the first three groups, but decreased when the number of CD4+ T-cells fell under 100 cells/μl blood (Fig. 2B).

The strongest differences in virus isolation results

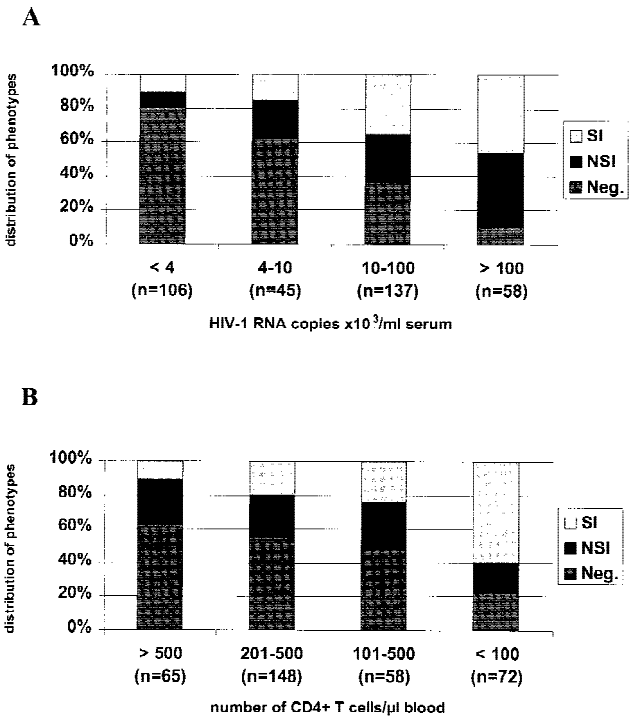


Fig. 2. Correlation of HIV-1 phenotypes with the amount of HIV RNA titer (**A**) and CD4+ T-cell count (**B**). With increasing viral load, the frequency of successful virus isolation rises significantly, whereas the ratio of isolated NSI to SI virus remains relatively constant (A). In contrast, the ratio of isolated NSI to SI virus decreases in association with decreasing CD4+ T-cells. Neg. denotes negative virus culture; NSI, nonsyncytia-inducing virus; SI, syncytia-inducing virus (B).

were observed when all three parameters were taken together. The highest detection rate (96.6%) of HIV in cell culture was achieved in patients with CD4+ T-cell counts below 100 cells/μl and HIV RNA titers above $10^5$. In contrast, in 40 patients with more than 500 CD4+ T-cells and less than $10^4$ HIV RNA copies/ml, virus isolation only succeeded in eight (20%) cases, including only one (2.5%) sample harboring SI virus in the PBMC.

Longitudinal investigations were carried out in order to monitor the changes in viral load, CD4+ T-cell count, and viral phenotype (Table II). In five patients the switch was observed from negative virus culture to NSI phenotype concomitant with significant increases in viral load (> 0.3 log; $P < 0.05$). In another patient, the opposite was observed, i.e., a decrease in viral RNA titer from 49,000 copies/ml to < 4,000 copies/ml was correlated with a switch from NSI isolate to negative virus isolation. In this patient, treatment with a AZT/3TC combination was initialized 1 month prior to the second investigation.

## DISCUSSION

The study clearly demonstrates that isolation and phenotyping of HIV represents a striking predictive marker for progression of HIV infection. Negative virus culture was associated with a significantly longer survival time when compared to patients presenting NSI

TABLE II. Changes of Viral Phenotype, Viral Load, and $CD4^+$ T-Cell Count in Six HIV-1–Infected Patients

| Patient | Date | Virus cultivation | HIV RNA copies × $10^3$/ml | $CD4^+$ T-cell count/ μl blood |
|---|---|---|---|---|
| A | 5/1994 | Negative | 32 | 293 |
| A | 10/1995 | NSI | 300 | 285 |
| B | 2/1995 | Negative | <4 | 401 |
| B | 6/1996 | NSI | 21 | 330 |
| C | 10/1994 | Negative | 6 | 173 |
| C | 7/1995 | NSI | 85 | 116 |
| D | 12/1994 | Negative | 26 | 37 |
| D | 11/1995 | NSI | 140 | 12 |
| E | 12/1994 | Negative | <4 | 380 |
| E | 8/1995 | NSI | 31 | 408 |
| F | 10/1994 | NSI | 49 | 87 |
| F | 10/1995 | Negative | <4 | 74 |

isolates. Moreover, the group of patients with SI virus displayed the highest mortality. Even the different grades of cytopathogenicity within the SI phenotype subgroup determined the survival time. Isolation of highly cytopathogenic SI variants was associated with the highest mortality, whereas patients with moderately cytopathogenic virus had a more favorable prognosis [Rockstroh et al., 1996]. Since Koot et al. [1993b] as well as Richman et al. [1994] found that the emergence of SI virus preceded the decline of CD4+ T-cells, followed by rapid progression to AIDS, the highly cytopathogenic phenoptype of HIV seems not to be the consequence, but one of the causative factors for the progression to AIDS.

The predictive value of virus cultivation is only partially in agreement with the other two major prognostic parameters, namely CD4+ T-cell count and viral load. With increasing amounts of HIV RNA copies in the patient's plasma, the rate of successful virus isolation increases significantly, but the ratio of NSI to SI isolates remains more or less constant. These data were confirmed when longitudinal samples were investigated. Moreover, with decreasing CD4+ T-cell count there is a significant increase of successful virus isolation, but in this case the ratio between negative isolation and isolation of NSI variants remains constant. This means that the progression from negative virus culture to the isolation of NSI variants is reasonably associated with an increase of viral load in the plasma but seems not to affect the amount of the CD4+ T-cells. A decrease of the T4 helper cells, however, is chronologically correlated with the shift from the NSI phenotype to the SI phenotype in PBMC culture [Fouchier et al., 1995].

Since in vitro, SI variants usually produce higher titers than NSI variants [Asjö et al., 1986], it may seem surprising that in vivo the NSI/SI phenotype does not influence the viral load. Other researchers could also demonstrate that a shift from the NSI to the SI phenotype was not accompanied with significant increases of HIV RNA in plasma [Juriaans et al., 1994; Katzenstein et al., 1996]. This phenomenon can partially be explained by the fact that the in vitro conditions of lymphocytes are not completely comparable to the situation in vivo. High nonspecific stimulation of PBMC by addition of phythemagglutinin and interleukin-2 to the culture medium activates viruses from latently infected cells, which might not attribute to the HIV-1 plasma RNA.

The currently proposed highly active antiretroviral therapy (HAART) is monitored by quantification of HIV-1 RNA and CD4+ T-cells. However, these two parameters of HIV-1 infection do not completely reflect the stage of disease. The results of virus cultivation attempts together with subsequent characterization of the isolated virus isolates proved to be of high prognostic value. Further studies are to be focused on the question as to whether a reverse evolution from SI virus to NSI or nonculturable virus occurs under HAART and whether such an event eventually influences the prognosis for the infected patients.

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